MOSH AND MOAH IN EDIBLE OILS AND FATS: TOXICOLOGIC AND ANALYTICAL ISSUES, SOURCES OF CONTAMINATION

Analysis of MOH in edible oils: Experience of SGS Institut Fresenius

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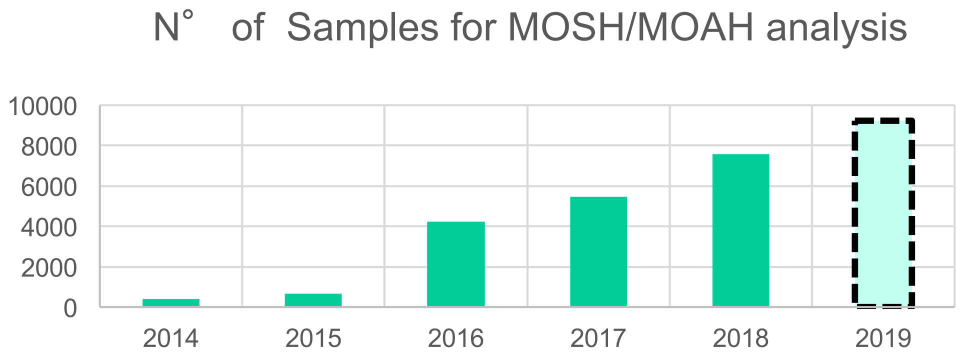
MOSH/MOAH determination at SGS

MOSH/MOAH laboratory at SGS Institut Fresenius GmbH, Berlin, Germany

SGS sites in Germany

2011-2013: **MOSH/MOAH method set-up**

2019:
7 FTE
4 GC-LC/FID
1 LC-GCxGC-TOF/MS

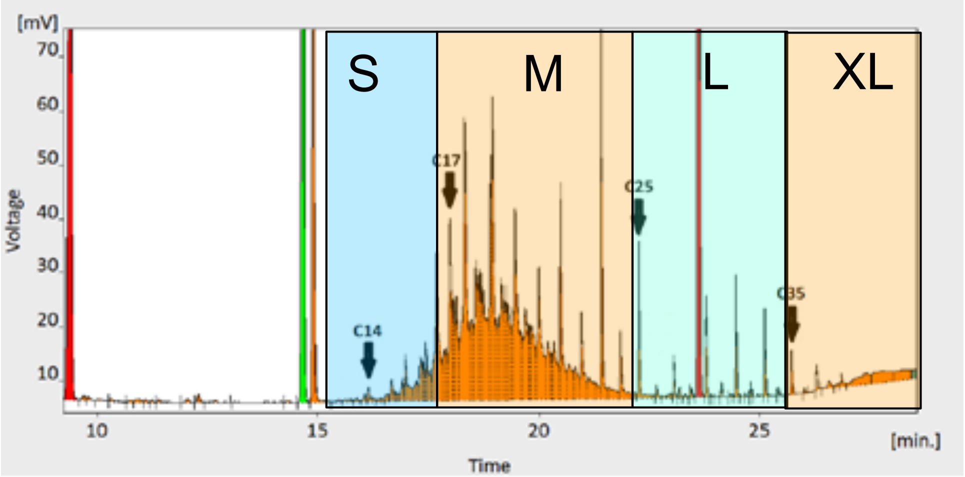


Edible oils & fats, easy extractable oil/fat-containing foods, infant formulae, various kinds of foods with low fat contents, food contact materials

General analytical approach for MOSH/MOAH determination

On-line coupled HPLC-GC-FID

1. **Analyte extraction from compound foods.**
2. **If necessary: epoxidation and/or column chromatography (silica/alox) for clean-up.**
3. **HPLC: matrix removal & separation of MOSH, MOAH.**
4. **Automated transfer of MOSH and MOAH fractions into GC-FID.**
5. **Parallel GC measurement of both fractions on two columns.**
6. **Integration of analyte humps** (UCM: unresolved complex mixture), **cut off of spikes, quantification of segments according to C-numbers on base of a set of internal standards.**



ISO 17780

Determination of aliphatic hydrocarbons in **vegetable oils**.

LOQ MOSH: 50 mg/kg

DIN EN 16995:2017-08

Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis in **vegetable oils and foodstuff on basis of vegetable oils**.

LOQ MOSH: 10 mg/kg / LOQ MOAH: 10 mg/kg

Validation data for MOSH/MOAH determination at SGS

Field of application: edible oils/fats

Repeatability (2015): RSRr MOSH: 13 % / RSDr MOAH: 16 % palm oil
Repeatability (2015) 10-12/3.1-4.6ppm: RSRr MOSH: 10 - 20 % / RSDr MOAH: 17 % hazelnut oil

Linearity (2015) 0 – 38,5/10,5 ppm: MOSH: 0.998 / MOAH: 0.997 in palm oil
Linearity (2018) 1 – 5/4,5 ppm: MOSH: 0.9996 / MOAH: 0.9993 in palm oil

LOD: does not apply (positive findings < LOQ not reported)

LOQ: MOSH = 1 mg/kg / MOAH = 1 mg/kg

Reproducibility (if any): sometimes poor at the low-ppm-level – see interlaboratory comparisons

Limitations of the current methodology

Several sample preparation procedures might be applied and:
Every laboratory goes it´s own way.

1) Various fat/analyte extraction procedures for compound foods.
2) Maybe oil/fat saponification
3) Maybe epoxidation
Strong or mild, EtOH or DCM
4) Maybe aluminium oxide column chromatography
manually or on-line
5) Maybe silica gel column chromatography
before or after epoxidation
6) Different strategies for raw data processing/reporting

Limitations of the current methodology

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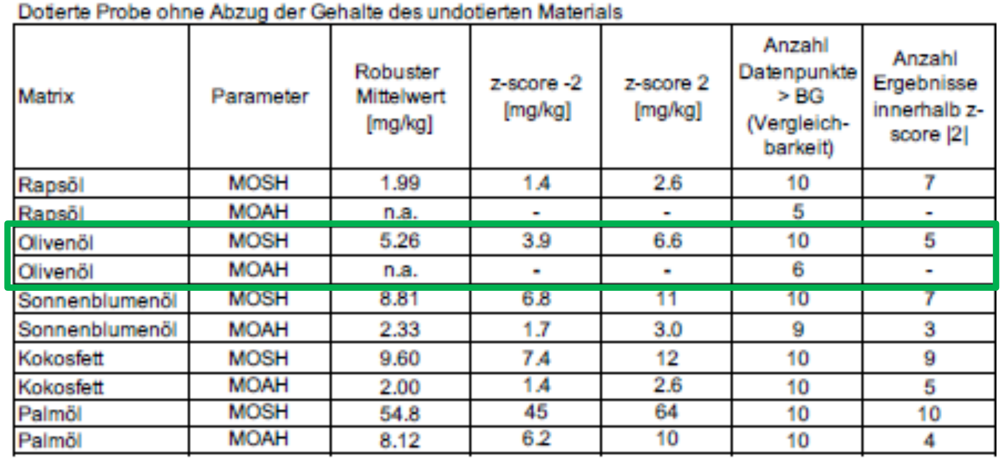
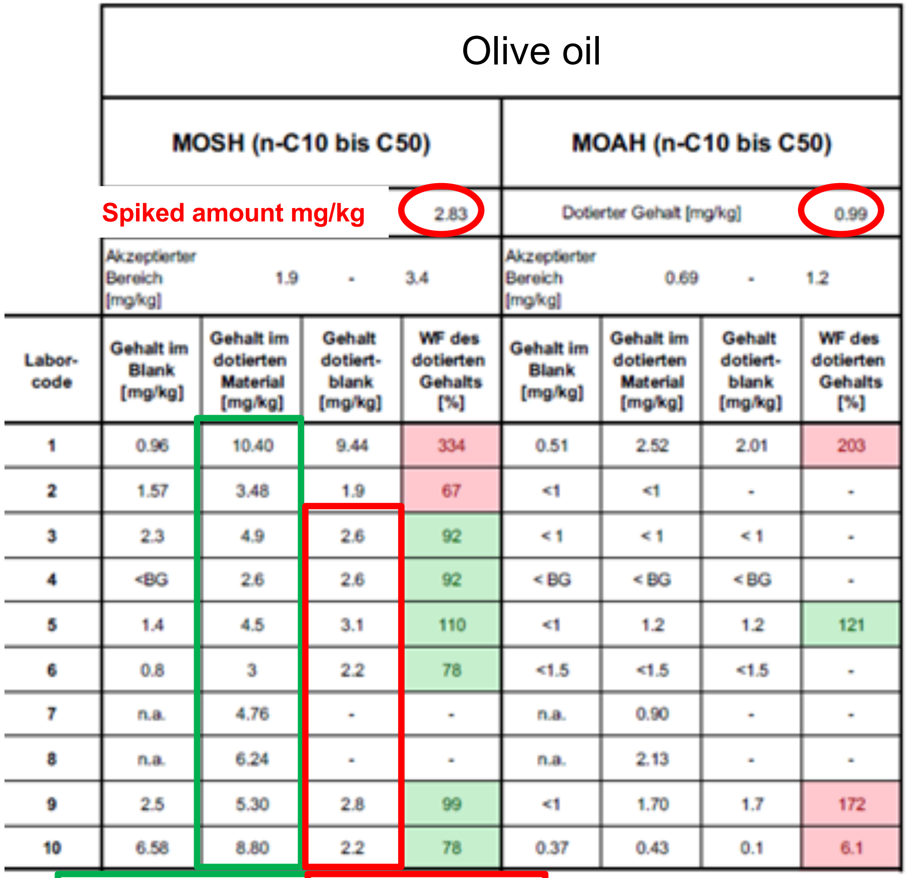


Dotierte Probe ohne Abzug der Gehalte des undotierten Materials

Matrix	Parameter	Robuster Mittelwert [mg/kg]	z-score -2 [mg/kg]	z-score 2 [mg/kg]	Anzahl Datenpunkte > BG (Vergleich-barkeit)	Anzahl Ergebnisse innerhalb z-score |2|
Rapsöl	MOSH	1.99	1.4	2.6	10	7
Rapsöl	MOAH	n.a.	-	-	5	-
Olivenöl	MOSH	5.26	3.9	6.6	10	5
Olivenöl	MOAH	n.a.	-	-	6	-
Sonnenblumenöl	MOSH	8.81	6.8	11	10	7
Sonnenblumenöl	MOAH	2.33	1.7	3.0	9	3
Kokosfett	MOSH	9.60	7.4	12	10	9
Kokosfett	MOAH	2.00	1.4	2.6	10	5
Palmöl	MOSH	54.8	45	64	10	10
Palmöl	MOAH	8.12	6.2	10	10	4



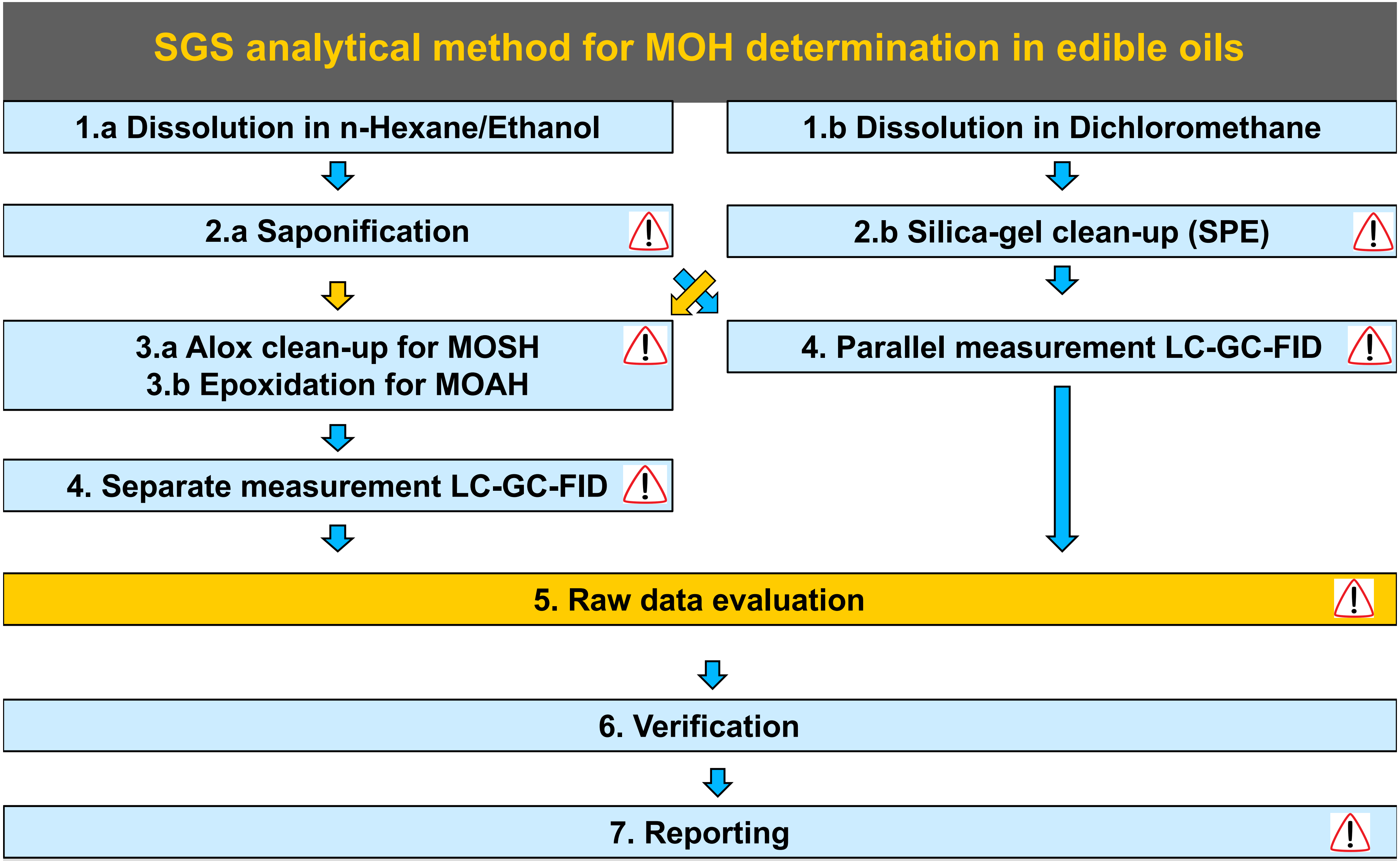
Olive oil

	MOSH (n-C10 bis C50)				MOAH (n-C10 bis C50)			
	Spiked amount mg/kg			2.83	Dotierter Gehalt [mg/kg]			0.99
	Akzeptierter Bereich [mg/kg]	1.9	-	3.4	Akzeptierter Bereich [mg/kg]	0.69	-	1.2
Labor-code	Gehalt im Blank [mg/kg]	Gehalt im dotierten Material [mg/kg]	Gehalt dotiert-blank [mg/kg]	WF des dotierten Gehalts [%]	Gehalt im Blank [mg/kg]	Gehalt im dotierten Material [mg/kg]	Gehalt dotiert-blank [mg/kg]	WF des dotierten Gehalts [%]
1	0.96	10.40	9.44	334	0.51	2.52	2.01	203
2	1.57	3.48	1.9	67	<1	<1	-	-
3	2.3	4.9	2.6	92	< 1	< 1	< 1	-
4	<BG	2.6	2.6	92	< BG	< BG	< BG	-
5	1.4	4.5	3.1	110	<1	1.2	1.2	121
6	0.8	3	2.2	78	<1.5	<1.5	<1.5	-
7	n.a.	4.76	-	-	n.a.	0.90	-	-
8	n.a.	6.24	-	-	n.a.	2.13	-	-
9	2.5	5.30	2.8	99	<1	1.70	1.7	172
10	6.58	8.80	2.2	78	0.37	0.43	0.1	6.1

Rob. mean: 5.26 mg/kg

mean: 2.58 mg/kg

SGS method



SGS analytical method for MOH determination in edible oils

1.a Dissolution in n-Hexane/Ethanol

2.a Saponification ⚠

3.a Alox clean-up for MOSH ⚠
3.b Epoxidation for MOAH

4. Separate measurement LC-GC-FID ⚠

1.b Dissolution in Dichloromethane

2.b Silica-gel clean-up (SPE) ⚠

4. Parallel measurement LC-GC-FID ⚠

5. Raw data evaluation ⚠

6. Verification

7. Reporting ⚠

Analytical pitfalls

Analytical method limitations

In terms of mineral oil trace determination in edible oils, sample preparation steps might be the source of significant uncertainty or error!

Consequently, the single working steps should be checked…
…for fitness of purpose!
…for harmonisation!

SGS method

Matrix removal by solvent distribution - saponification

2.a Saponification & extraction
2.aa Alkaline digestion: sample in nH/EtOH + int. Stds + KOH/ 30 min/60° C.
2.ab Lipid/unsaponifiable extraction: l/l-extraction with *n*-hexane.

Application of saponification has a significant impact on the matrix-load of samples and might support efficiency of subsequently applied clean-up steps, especially epoxidation

- Every sample preparation step might result in
  losses of analytes (risk of underestimation)
  carry-over/contamination (risk of overestimation/false positives).

SGS suggestion in perspective of method harmonisation:

- In depth testing of suitable methods for saponification and extraction of the unsaponifiable.

SGS method

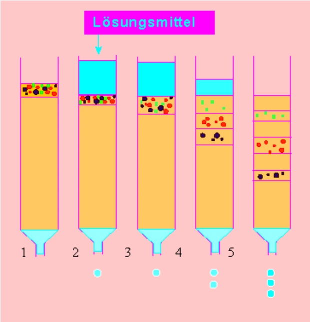
Matrix removal by silica-gel column chromatography

(solid-phase-extraction = SPE)

2.b SPE: silica-gel column (3 g, 70 – 230 mesh).

Elution with 20 mL CH_2Cl_2/n-Hexane, addition of keeper, controlled solvent evaporation. Transfer into 400 µL *n*-Hexane.

SPE serves for sample clean-up. Biogenic matrix components and other polar substances retain on the column.



- Silica-SPE is a standard procedure that can be carried out in reproducible manner.
  - However, changes in nature or amount of elution solvent, elution speed, silica amount/particle size/deactivation degree, or variation of the column geometry might have a negative impact analyte recoveries.
  - Overdone solvent evaporation might cause losses of volatile analytes.

SGS suggestion in perspective of method harmonisation:

- Agreement on the **exact** Silica-SPE procedure.

SGS method

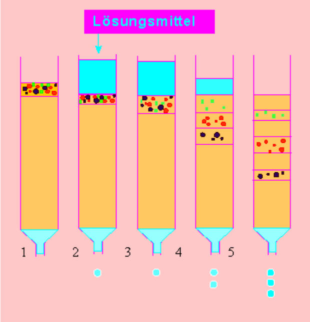
Matrix removal by silica-gel/aluminium oxide column chromatography

(solid-phase-extraction = SPE)

3.a SPE: silica-gel/alox column (3 g/10g, 70 – 230 mesh/0.06-0.2mm cond.).

Elution with 20 mL CH_2Cl_2/n-Hexane, addition of keeper, controlled solvent evaporation. Transfer into 400 µL *n*-Hexane.

„Alox-SPE“ serves for MOSH sample clean-up (removal of biogenic n-alkalnes)



- ➢ SPE is a standard procedure that can be carried out in reproducible manner.
- ➢ However, changes in nature or amount of elution solvent, elution speed, silica amount/particle size/deactivation degree, or variation of the column geometry might have a negative impact analyte recoveries.
- ➢ Overdone solvent evaporation might cause losses of volatile analytes.

SGS suggestion in perspective of method harmonisation:

- ➢ Agreement on the **exact** Alox-SPE procedure for MOSH determination.

SGS method

MOAH: Matrix removal by epoxidation

3.b Epoxidation: Purified *m*-CPBA in ethanol to extract **2.**, vortex 15 min @ 40 ° C.
Reaction stop with $Na_2S_2O_3$, mixture remains 5 min @ RT.

⚠

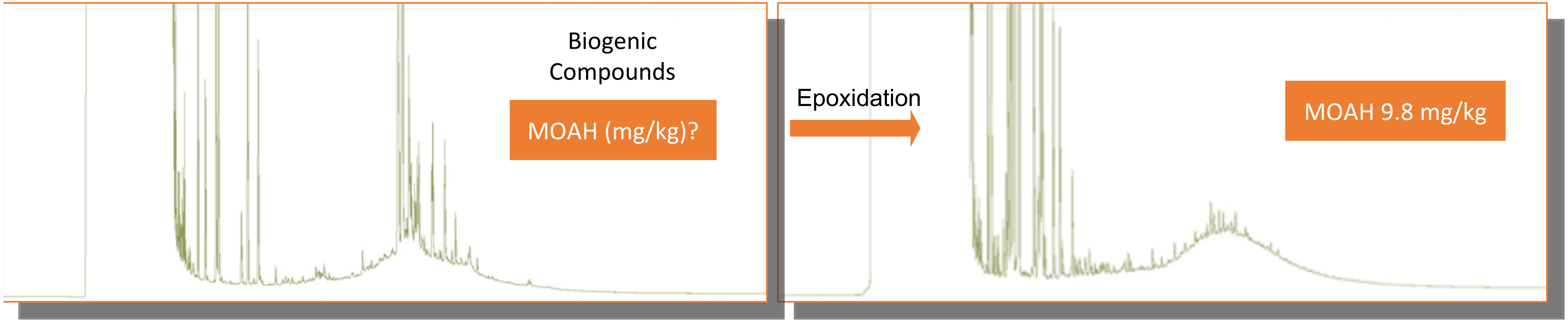
↓

Epoxidation serves for preparation of removal of interfering components. Functional groups such as reactive double bonds will be converted into polar oxiranes.

However, it is a harsh chemical reaction which is difficult to be quantitatively controlled.

SGS method

Matrix removal by epoxidation



- ➢ Not applying epoxidation might result in analyte overestimation due to contribution of non-aromatic components to the MOAH-hump.
- ➢ But, epoxidation also causes losses of MOAH: Risk of underestimation.

SGS suggestion in perspective of method harmonisation:

- ➢ General decision **when** to apply epoxidation.
- ➢ Agreement on the **exact** epoxidation procedure.

SGS method

Instrumentation

4. Measurement: Automated LC-GC-FID standard equipment. H_2 as carrier gas.

2 GC-columns for parallel MOSH/MOAH determination.

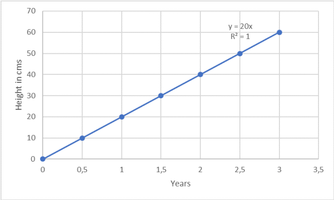
⇩

HPLC serves for matrix removal and separation of MOSH and MOAH.
GC allows separation of the analytes in dependency upon Carbon-numbers.

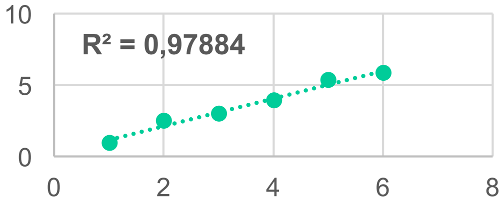
So far the most suitable and practical solution – widely applied.

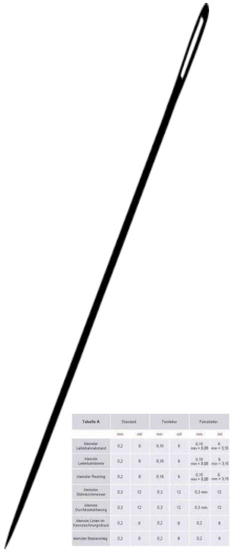
- ➢ FID is (desirably) non-selective. But it is not very sensitive.
- ➢ Pro: excellent linearity and signal/dose correlation independent upon nature of the analytes.
- ➢ Con: not first choice for trace analysis, gives no information on analyte structures.

GC-FID



GC²-TOF-MS





SGS suggestion in perspective of method harmonisation:

- ➢ LC-GC-FID seems to be the best choice for reproducible quantitation.
- ➢ Demands for LOQ should be realistic – otherwise official method validation might fail.

SGS method

Data processing

5. Raw data evaluation: Integration of MOSH/MOAH analyte humps.

Spike-cut-off, quantification of 6/4 segments according to C-numbers on base of a set of iStds.

MOSH: < C16 ↔ C20 ↔ C25 ↔ C35 ↔ C40 ↔ C50

MOAH: < C16 ↔ C25 ↔ C35 ↔ C50

⇩

As no individual target analytes can be ressolved by GC-FID, MOH are detected as UCM. Standard quantitation software allows **integration of hump segements** and **cut-off of spikes** originating from biogenic components.

This seems to be the only feasible approach – but a lot of experience and good chromatographic conditions are required.

- Non-standardized integration protocolls and changes in the instrumental or personal performance might cause increased measurement uncertainty, especially at the low concentration level.
- Underlying components being of non-petrochemical origin might cause overestimations.

SGS suggestion in perspective of method harmonisation:

- Agreement on the **exact** procedure of data processing.

SGS method

Result verification

6. Verification: Check for MOSH/MOAH-relation, occurrence of fossile marker molecules, hump symmetry *et cetera*. In case MOAH positive: GC x GC-TOF-MS analysis.

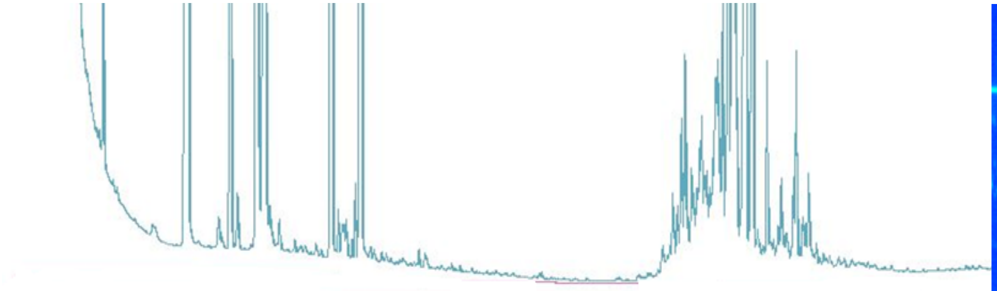
- MOSH : MOAH realtions might give important information in terms of verification.

- GC²-TOF-MS analysis allows the separation and identification of different substance classes from which the GC-FID-UCM consists.

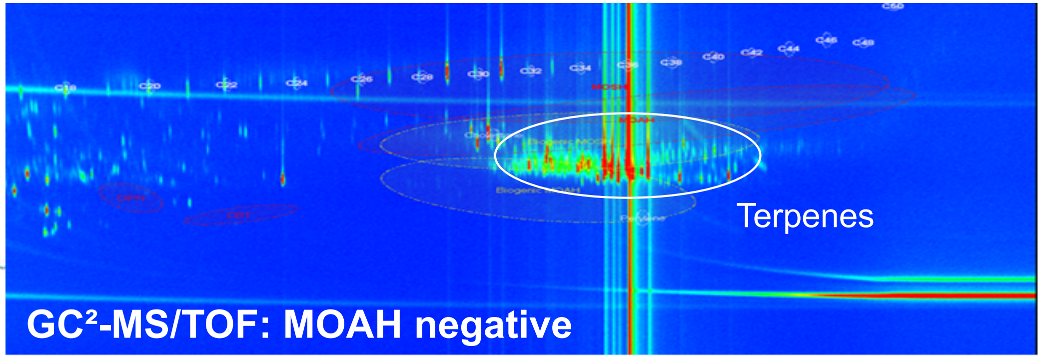
- Not considering MOSH-results for verification of MOAH findings might be a wasted opportunity.
- GC²-TOF-MS: Expensive equipment. Personel has to be well-educated and trained to run the instruments and to interprete the data.
- Quantitation by GC²-TOF-MS seems to be not feasible – so far.

SGS method

Result verification



GC-FID: MOAH positive



GC²-MS/TOF: MOAH negative

SGS suggestion in perspective of method harmonisation:

- In terms of expenses and effort, it seems to be difficult to include expensive and highly sophisticated but non-quantitative techniques into method harmonisation.

„Dilemma“

- On the other hand, the trueness of MOAH-results might remain questionable without application of more sophisticated techniques!

- Some advice, how to verify results in a quantitative manner would be desirable with a harmonised method!

SGS method

Reporting

7. Reporting: results reported in mg/kg sample after **background substraction**.
LOQ = 1 $mg/kg_{\textbf{lower bound}}$ per fraction and in sum

- Lower-bound approach according to common procedure for sum parameters (e.g. pesticides, pyrrolizidine alkaloids etc.)

from our perspective justified by the assumption that the risk for false-positive might be higher as the risk of false-negative.

- Same sample might give significantly different results in case of medium-bound or upper-bound application.
- In case of upper-bound, the corresponding LOQ for the sum of MOSH or MOAH would be increased accordingly.
- Maybe best choice to integrate the complete hump.

SGS suggestion in perspective of method harmonisation:

- Agreement on target-LOQs especially for different matrices.
- Agreement on a general procedure to sum up the results of the single MOAH fractions.

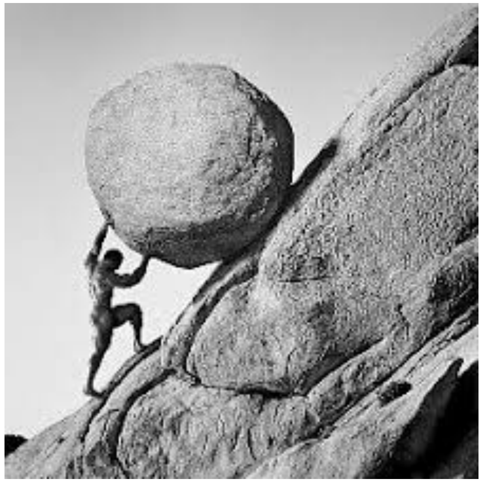
SGS method

Outlook: Method improvements in preparation

SGS works on a GC²-TOF-MS/FID method to combine the superior chromatographic resolution of two-dimensional GC with TOF-MS structure information in combination with the excellent signal/dose correlation of the FID.

GC-FID ⟷ **GC²-MS/TOF** ⟷ **GC²-TOF-MS/FID**

- However, this solution might improve the trueness of the MOSH/MOAH-determination – but it does not automatically improve sensitivity, measurement uncertainty or reproducibility.



Conclusions

Take-home-messages

- In course of MOSH/MOAH analysis, edible oils/fats represent a challenging matrix.
- Common MOH analysis is based on several sample preparation steps which might be the source of error.
- SGS would favorite to apply MOSH/MOAH determination in edible oils/fats in a fully harmonized way.
- From our perspective, enhanced analytical techniques for better analyte resolution and structure identification have to be established for common application in order to avoid possible MOH-overestimations or false-positive findings.
- SGS also estimates, that official method validation for MOH-determination in the very low ppm-range might be not feasible with the current methodology – as long as the impact of the single sample preparation steps on reproducibility hasn´t checked systematically.